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1: Prostaglandins Leukot Essent Fatty Acids. 2000 May;62 (5):319-27.

Relationship between the ability to support differentiation of osteoclast-like cells and adipogenesis in murine stromal cells derived from bone marrow.

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In vitro osteoclast differentiation is supported by stromal cells. In order to isolate a stromal cell line that can support osteoclast differentiation, 22 cell lines were cloned from mouse bone marrow. One of these clones, TMS-14, is a line of preadipocytes that supports osteoclast-like cell formation without any bone resorbing factors; and another, TMS-12, is a line of preosteoblasts that supports osteoclast-like cell formation with bone resorbing factors such as prostaglandin E(2)(PGE(2)). The difference of these two lines for osteoclast formation was not related with their abilities of PGE(2)production, but with the expression of osteoclast differentiation factor (ODF, also called OPGL, RANKL, and TRANCE), which detected with RT-PCR, in both cell lines. In TMS-14 cells, ODF mRNA was detected with or without PGE(2). In TMS-12 cells, ODF expression was detected in the PGE(2)-treated cells alone. When TMS-14 cells were induced to undergo adipogenic differentiation in response to treatment with thiazolidinedione, a ligand and activator of peroxisome proliferator-activated receptor gamma (PPARgamma), the ability of TMS-14 cells to support osteoclast-like cell formation was prevented in the presence or absence of 1,25(OH)(2)D(3). The gene expression of ODF in TMS-14 cells was also inhibited by treatment with thiazolidinedione. These results suggest that adipogenesis in bone marrow cells is related to the ability to support osteoclast differentiation. This is the first report of a cloned stromal cell line that can support osteoclastogenesis without the treatment with any osteotropic factors. Furthermore, this murine clonal preadipose cell line may be useful for studying senescence-dependent osteoporosis. Copyright 2000 Harcourt Publishers Ltd.

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Evidence that the rat osteopetrotic mutation toothless (tl) is not in the TNFSF11 (TRANCE, RANKL, ODF, OPGL) gene.

Odgren PR, Kim N, van Wesenbeeck L, MacKay C, Mason-Savas A, Safadi FF, Popoff SN, Lengner C, van-Hul W, Choi Y, Marks SC Jr.

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The toothless (tl) osteopetrotic mutation in the rat affects an osteoblast-derived factor that is required for normal osteoclast differentiation. Although the genetic locus remains unknown, the phenotypic impact of the tl mutation on multiple systems has been well characterized. Some of its actions are similar to tumornecrosis factor superfamily member 11(TNFSF11; also called TRANCE, RANKL, ODF and OPGL) null mice. TNFSF11 is a recently described member of the tumor necrosis factor superfamily which, when expressed by activated T cells, enhances the survival of antigen-presenting dendritic cells, and when expressed by osteoblasts, promotes the differentiation and activation of osteoclasts. The skeletal similarities between tl rats and TNFSF11(-/-) mice include 1) profound osteoclastopenia (TNFSF11-null mice, 0% and tl rats 0-1% of normal); 2) persistent, non-resolving osteopetrosis that results from 3) a defect not in the osteoclast lineage itself, but in an osteoblast-derived, osteoclastogenic signal; and 4) a severe chondrodysplasia of the growth plates of long bones not seen in other osteopetrotic mutations. The latter includes thickening of the growth plate with age, disorganization of chondrocyte columns, and disturbances of chondrocyte maturation. These striking similarities prompted us to undertake studies to rule in or out a TNFSF11 mutation in the tl rat. We looked for expression of TNFSF11 mRNA in tl long bones and found it to be over-expressed and of the correct size. We also tested TNFSF11 protein function in the tl rat. This was shown to be normal by flow cytometry experiments in which activated, spleen-derived T-cells from tl rats exhibited normal receptor binding competence, as measured by a recombinant receptor assay. We also found that tl rats develop histologically normal mesenteric and peripheral lymph nodes, which are absent from TNFSF11-null mice. Next, we found that injections of recombinant TNFSF11, which restores bone resorption in null mice, had no therapeutic effect in tl rats. Finally, gene mapping studies using co-segregation of polymorphic markers excluded the chromosomal region containing the TNFSF11 gene as harboring the mutation responsible for the tl phenotype. We conclude that, despite substantial phenotypic similarities to TNFSF11(-/-) mice, the tl rat mutation is not in the TNFSF11 locus, and that its identification must await the results of further studies.

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The TNF receptor superfamily: role in immune inflammation and bone formation.

Cheng X, Kinosaki M, Murali R, Greene MI.

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Tumor necrosis factor (TNF) and TNF receptor (TNFR) family proteins play important roles in many biological processes. Recently, the TNF-family molecule, RANKL (also called TRANCE, ODF, and OPGL), and its receptors, RANK and OPG, were found to be regulators of the development and activation of osteoclasts in bone remodeling. TNFalphaalso activates osteoclasts both by themselves and in synergy with RANKL. We used structure-based design to create peptidomimetics and organic therapeutics that inhibit osteoclastogenesis by inhibiting the interaction of ligands and receptors. Here we show for the first time that blocking TNFalpha by these small molecules effectively inhibited osteoclast formation in vitro. These mimetics can be used as a probe to understand the molecular basis of osteoclastogenesis and also as a platform to create useful therapeutic agent.

Publication Types:
- Review
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PMID: 12857975 [PubMed - indexed for MEDLINE]

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Perspective. Osteoclastogenesis and growth plate chondrocyte differentiation: emergence of convergence.

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A "bone" is really a dynamic and highly interactive complex of many cell and tissue types. In particular, for the majority of skeletal elements to develop and grow, the process of endochondral ossification requires a constantly moving interface between cartilage, invading blood vessels, and bone. A great deal has been learned in recent years about the regulation of chondrocyte proliferation and differentiation by hormones, growth factors, and physiologic stimuli during skeletal development and growth. Likewise, the discovery that colony stimulating factor-1 (CSF-1, or M-CSF) and receptor activator of NF-kappaB ligand (RANKL, a tumor necrosis factor superfamily member also called TRANCE, ODF, OPGL, and TNFSF11) are pivotal in communicating from osteoblasts to osteoclasts has led to deeper insights into bone growth, turnover, and maintenance. Little is known, however, about how these two quite different systems communicate to solve the problem of providing integrated, continuous mechanical support during the dynamic invasion of cartilage by bone that characterizes endochondral bone growth. Evidence has accumulated in recent years that provides insight into the communication between growing bone and cartilage in the form of a subset of osteopetrotic mutations, which share a lack of osteoclasts and an accompanying chondrodysplasia of the growth plate. These mutations thus implicate some of the same gene products in regulating chondrocyte differentiation and bone resorption. We also consider expression studies of some known growth plate regulators, such as parathyroid hormone-related protein (PTHrP) and Indian hedgehog (Ihh), in light of this and propose a model in which the osteoclastogenic factors act also on chondrocytes, but downstream of PTRrP and Ihh in regulating proliferation and differentiation, and after early morphogenic patterns are established.

Publication Types:
- Review
- Review, Tutorial

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1: Yi Chuan Xue Bao. 2004 Jul;31(7):675-81.

Expression, purification and bioactivity characterization of extracellular domain of murine osteoprotegerin ligand.

Wang BL, Qiu MC, Guo G, Liang DC, Zhang JY.

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Osteoprotegerin ligand (OPGL) is a key regulator of formation and activation of osteoclasts. In the present study, the cDNA encoding the extracellular domain of murine OPGL (sOPGL) was synthesized by RT-PCR and cloned into fusion expression vector pET-42a(+) in a certain strategy on purpose that the fusion tag could be completely removed by factor Xa from the expressed fusion protein without any vector-encoded sequence left. Induced with IPTG, the recombinant E. Coli cells produced a 47 kD protein in high level that could be recognized, through Western blotting analysis, by the antibody against OPGL. The expressed products were purified through Glutathione-sepharose 4B affinity chromatography. Along with the fusion molecule, a protein about 30 kD was also specifically bound to the resin. The 30 kD molecule could be recognized by polyclonal antibody against GST-IGF-1, but not by antibody against OPGL. It suggested that the 30 kD molecule was derived from the degradation of the fusion protein. After the cleavage with factor Xa and further purification, the fusion tag was removed and the recombinant sOPGL was obtained. Finally, we confirmed that the recombinant sOPGL could promote osteoclast formation from mouse bone marrow cells in a dose dependent manner.

PMID: 15473318 [PubMed - indexed for MEDLINE]

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1: J Lab Clin Med. 2004 Oct;144(4):193-200.

Gene expression in giant-cell tumors.

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Malignant transformation is thought to be associated with changes in the expression of a number of genes, and this alteration in gene expression is considered critical to the development of the malignant phenotype. In this study, gene expression in 8 samples of giant-cell tumor (GCT) of bone, as well as in bone at the site of osteoarthritis and in a variety of normal tissues, was determined at Gene Logic Inc (Gaithersburg, Md) with the use of Affymetrix GeneChip U_133 arrays containing approximately 40,000 genes/expressed sequence tags (ESTs). Gene-expression analysis was performed with the use of the Gene Logic GeneExpress Software System. Differences in gene expression between GCTs and bone were observed. In addition, genes expressed uniquely in GCTs among these and 519 samples from 20 other tissue types were identified. Some of the genes that were found to be overexpressed in GCTs, such as tartrate-resistant acid phosphatase and the lysosomal H + -transporting ATPase, are also expressed by osteoclasts. Osteoprotegrin ligand (OPGL) was also selectively overexpressed in GCTs. The genes found to be overexpressed in GCTs appear to reflect the genetic profile of osteoclast-lineage cells and also the genetic profile of an osteoclastogenic environment. The genes identified in this study may play a role in the pathogenesis of GCTs, confirm the likely importance of OPGL in GCT pathogenesis, and may indicate other possible targets to which antitumor therapy could be directed.

PMID: 15514587 [PubMed - indexed for MEDLINE]

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Ratio of cyclase activating and cyclase inactive parathormone (CAP/CIP) in dialysis patients: correlations with other markers of bone disease.

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PURPOSE: We checked correlation of CAP/CIP with osteoprotegrin (OPG), its soluble ligand (OPGL) and routinely measured parameters of bone turnover in patients treated with peritoneal dialysis (PD) and hemodialysis (HD). MATERIAL & METHODS: In 30 patients (22 HD, 8 PD) we determined serum concentrations of intact parathormone (iPTH), CAP, OPG, OPGL, total Ca, inorganic phosphates (Pi), creatinine, urea, total alkaline phosphatase (AP) and blood pH. CIP was calculated by subtraction of CAP from iPTH. Controls (Cs) included 9 healthy persons in whom iPTH, CAP, OPG and OPGL were measured as well as CIP, CAP/CIP and OPGL/OPG were calculated. RESULTS: Differences between HD and PD patients included dialysis duration, OPGL, OPGL/OPG, AP, Pi, Ca and pH. After adjustment to dialysis duration differences in OPGL/OPG, Pi, Ca and pH remained significant. HD patients differed from Cs in terms of iPTH, CAP, CIP, OPGL, OPG and OPGL/OPG. In whole group of patients iPTH, CAP, CIP but not CAP/CIP correlated negatively with OPGL and OPGL/OPG as well as positively with dialysis duration, OPG and AP. CONCLUSIONS: Despite more advanced uremic bone disease in longer dialyzed HD patients than in shorter dialyzed PD ones, CAP/CIP is not different neither between these groups nor Cs persons. CAP/CIP does not seem to be more powerful tool in noninvasive diagnosis of bone disease than iPTH or CAP and CIP alone.

PMID: 15631341 [PubMed - in process]

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1: Arch Pharm Res. 2004 Dec;27(12):1258-62.

Rolipram, a phosphodiesterase 4 inhibitor, stimulates osteoclast formation by inducing TRANCE expression in mouse calvarial cells.

Cho ES, Yu JH, Kim MS, Yim M.

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Phosphodiesterase (PDE) 4 is an enzyme that degrades intracellular cAMP. In the present study, the effect of rolipram, a specific phosphodiesterase (PDE) 4 inhibitor, on osteoclast formation was investigated. Rolipram induced osteoclast formation in cocultures of mouse bone marrow cells and calvarial osteoblasts. This activity was not observed in the absence of calvarial osteoblasts, suggesting that calvarial osteoblasts are likely target cells of rolipram. Osteoclast formation by rolipram was completely blocked by the addition of osteoprotegerin (OPG), a soluble decoy receptor for the osteoclast differentiation factor, TNF-related activation-induced cytokine (TRANCE, identical to RANKL, ODF, and OPGL). Northern blot analysis revealed the effect of rolipram to be associated with the increased expression of TRANCE mRNA in mouse calvarial osteoblasts. Collectively, these data indicate that PDE4 inhibitor up-regulates the TRANCE mRNA expression in osteoblasts, which in turn controls osteoclast formation.

PMID: 15646801 [PubMed - in process]

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